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Accumulation, Fractionation and Release of Oil by the Intertidal Clam 'Macoma balthica'

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16. ABSTRACT

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Accumulation, Fractionation and Release of Oil by the Intertidal Clam *Macoma balthica**

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Abstract

The bivalve mollusc *Macoma balthica* accumulated hydrocarbons during 180 days of continuous exposure to Prudhoe Bay crude oil in seawater dispersions with nominal concentrations of 0.03, 0.3 and 3.0 mg l^{-1}. The mollusc's ability to concentrate oil from seawater increased with decreasing oil-in-water concentration. Decreases in oil burden began after 30 to 120 days (depending on the oil concentration) and continued for at least 60 days after exposure to oil ceased. Aliphatic and aromatic hydrocarbons were fractionated in markedly different ways by the bivalve. Branched and cyclic aliphatics in the molecular weight-range dodecane through hexadecane were preferentially retained over straight-chain and their higher homologs. Larger and more substituted aromatic compounds were selectively concentrated. There appeared to be no selective concentration of aromatic sulphur compounds.

Introduction

The rate of hydrocarbon accumulation in marine organisms upon exposure to sublethal concentrations of oil in seawater and the subsequent loss of those hydrocarbons after termination of oil exposure have been reviewed by Varanasi and Malins (1977). Some of this information has come from *in situ* observations after an oil spill or in a chronically polluted area, and other information has come from laboratory studies with static or flow-through systems. While much work remains to be done, one result of the oil exposure studies is clear: marine organisms, and in particular bivalves, have the ability to concentrate petroleum hydrocarbons from seawater in their tissues.

We have conducted a controlled, laboratory experiment in which the bivalve mollusc *Macoma balthica* was exposed to seawater-dispersed crude oil for 180 days. In this paper we describe the apparatus in which this experiment was conducted and report the accumulation, fractionation and release of oil by the test bivalves.

Materials and Methods

In order to provide a realistic simulation of chronic exposure of marine animals to oil-in-seawater dispersions in the laboratory, we constructed a continuous-flow system capable of simultaneously delivering dispersions with nominal oil in water concentrations of 0.03, 0.3 and 3.0 mg l^{-1}. Our system is based primarily on the design of Hyland *et al.* (1977). Because we wished to disperse a moderately viscous crude oil, we also incorporated some of the features of Roubal *et al.* (1977).

The components of the system, as shown in Fig. 1, are a constant-head pressure tank, a mixing and separation tank, a manifold to deliver seawater and the oil/seawater dispersion to each of 8 exposure tanks, and filter drums to remove the oil. Seawater was maintained at a constant level in the head tank (A in Fig. 1) and flowed into the mixing chamber (B) at 10 l min^{-1}. A peristaltic pump (D) meters Prudhoe Bay crude oil (C) into the mixing chamber at 0.3 ml min^{-1}. Mixing energy was applied by a stainless-steel blade rotated at high speed by a stirring motor (F). The oil/seawater dispersion separated during its passage through a 340 l tank (G) and was removed from below the resulting oil slick at 6.66 l min^{-1} as it entered the dosing manifold (I). The tanks used were constructed of marine plywood covered with fiberglass resin. All plumbing was done with rigid PVC pipe and fittings. The mixing chamber

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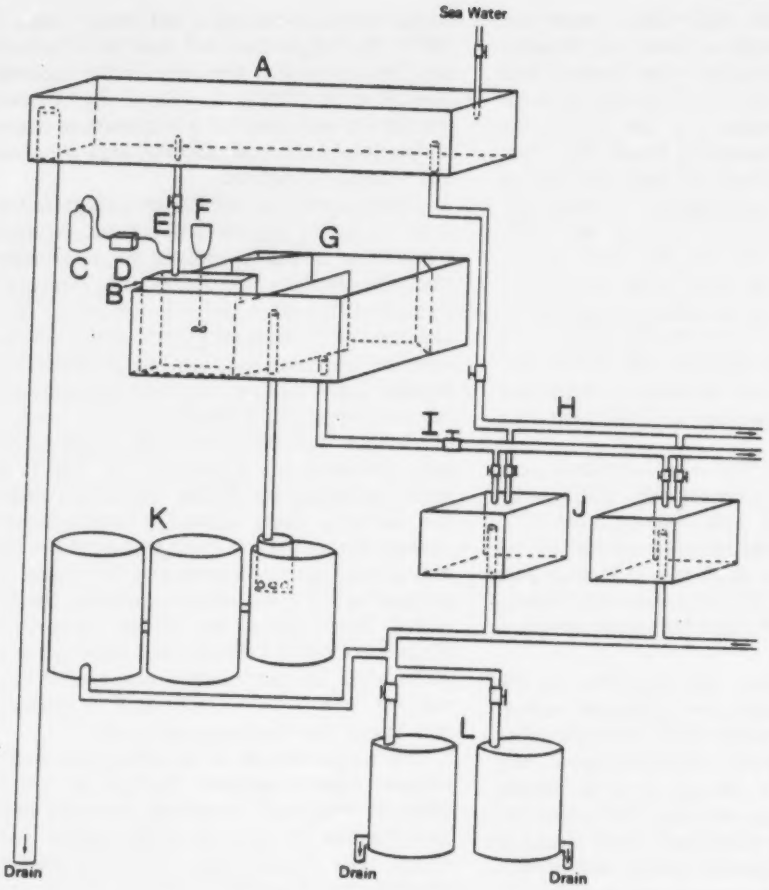

Fig. 1. Schematic representation of continuous oil-exposure system. A: head tank; B: mixing chamber; C: oil supply; D: oil pump; E: seawater supply; F: stirring motor; G: separation tank; H: non-contaminated seawater manifold; I: oil/seawater dispersion manifold; J: exposure tanks; K: separation tanks; L: final filter

was constructed of acrylic plastic. The entire system was leached with running seawater for 2 weeks prior to use. Evidence of contamination from plastics was not detected in either water or molluscs. Overflow from the separation tank passed through a plastic-fibre-floss filter (K), two 120 l separation drums and one of the two 80 l polyethylene drum filters (L) containing hydrophobic plastic strips, plastic floss and activated charcoal.

Different exposure concentrations were achieved by dilution of the oiled seawater with non-contaminated seawater (H) from the head tank to give a total flow of 3 l min^{-1} through each exposure tank. Seawater was supplemented with diatom-enriched seawater ($\triangleq$ 1:1 mixture) from "upwelling ponds" on the grounds of the Seward marine facility (Nevé *et al.*, 1976) for a period of 4 to 6 h per day beginning 9 July and ending 24 November 1977. The average exposure temperature was 8.0 °C (Range = 7.1 ° to 9.0 °C).

Specimens of *Macoma balthica* obtained from Resurrection Bay near Seward, Alaska (149°27'W; 60°06'N) were maintained in sand-filled petri-dish bottoms immersed in flowing seawater. Only clams which buried themselves were used for experimentation. On 14 June 1977, after 2 weeks acclimatation, the 180-day oil exposure experiment was begun. Water samples were collected from all exposure tanks and analyzed for hydrocarbon concentrations at 30-day intervals beginning on Day 0 and ending on Day 150. Replicate samples of approximately 50 specimens of *M. balthica* were obtained from each treatment periodically throughout the 180-day exposure period and one set of replicate samples from the control, low oil, and medium oil treatments after 60 days recovery in clean seawater. The clams were sieved from the silica sand, placed in fiberglass mesh buckets, and returned to their exposure tanks for 24 h. There was no food available at this time, allowing the clams to clear their intestinal tracts. After 24 h they were removed from the tanks, rinsed with clean seawater and immediately frozen.

Water samples were liquid-liquid extracted with three 5% (volume) hexane washes in a separatory funnel. The hexane extracts were combined, dried over Na_2SO_4, and reduced to 2 ml under vacuum in a rotary evaporator. The evaporation parameters were optimized for the recovery of napthalene. Clam-tissue samples were analyzed for hydrocarbons by modification of the method proposed by Warner (1976). Tissue was removed from the shell while still frozen and placed in a tared

50 ml centrifuge tube with teflon-lined screw-cap. Approximately 10 g wet weight of tissue was obtained from 50 *Macoma balthica*. Samples were digested with 10 N NaOH at 90° for 3 h, and allowed to cool to room temperature (ca. 20 °C). Hexane was then added, the tube was resealed, and then shaken vigorously for 2 min. The sample was then centrifuged at 2400 rpm for 10 min. The organic phase was subsequently removed with a 20 ml syringe and the procedure repeated twice. The hexane extract was dried over Na_2SO_4 and concentrated to between 1 and 2 ml on a rotary evaporator, and further concentrated to 0.5 ml under nitrogen before fractionation on a column of 5 g of 5% deactivated silica gel. A saturate hydrocarbon fraction was eluted with hexane and an unsaturated and aromatic hydrocarbon fraction with 40% benzene/hexane or 20% methylene chloride/hexane.

Quantification and identification of individual compounds was either by gas chromatography (GC) or by gas chromatography-coupled mass spectrometry (GC-MS). Samples were concentrated to approximately 0.2 ml and an aliquot injected onto a 30 m x 0.75 mm glass SCOT column coated with OV-101 in either an Hewlett-Packard (HP) 5710 GC with flame ionization detector, or an HP 5930/5933 GC-MS.

Compounds were identified and quantified on the GC-MS by single ion monitoring and calibration with an external standard. Aromatic compounds were quantified by reference to fully deuterated naphthalene and anthracene which were included in the sample as an internal standard. Alkylated aromatics were assumed to have the same response as the parent compound which results in an increasing but unknown underestimation with increasing alkyl substitution. The percentage recovery after the sample handling process was calculated from the recovery of an alkane, aromatic standard added to one of the two control samples from each sampling date before digestion. All hydrocarbon values are corrected for this handling loss, which averaged 25%.

Results

Total hydro[illegible]n concentration (mg oil l^{-1} seawater) for each exposure level measured at 30-day intervals is presented in Fig. 2. The greatest relative variability is encountered at the control and low oil levels. The overall pattern is that of high exposure level or undiluted mixing tank output. The increased variability of the lower oil levels is due to the widely varing control levels. Over 150 days, when the water hydrocarbon concentrations were measured (Fig. 2), the levels were relatively constant and an order of magnitude apart at nominal values of 0.03, 0.3, and 3.0 mg l^{-1}. Related work (Shaw and Reidy, 1979) has shown that 86% of the dispersed oil produced in this experiment was contained in particles larger than 5 μm.

The hydrocarbon concentrations in *Macoma balthica* tissue over the 180-day period of exposure to oil and the 60-day depuration period are presented in Fig. 3. [These results are expressed on a wet weight basis; those who prefer dry weight basis will find the information necessary for conversion tabulated in the succeeding paper (Stekoll *et al.* 1980)] As one of the two control clam samples for each date had a hydrocarbon standard added to determine analytical recovery, only the unaltered control values are presented.

Clams exposed to the highest concentration of oil in water (3.0 l^{-1}) rapidly accumulated hydrocarbons to a maximum of nearly 1500 $\mu g\ g^{-1}$ wet weight after 30 days. Their hydrocarbon content then gradually decreased throughout the rest of the exposure period. Initial uptake decreased with decreasing oil exposure levels; however, both at medium and low oil concentrations, clams reached their maximum hydrocarbon content after 120 days of exposure. Hydrocarbon levels in clams exposed to medium concentrations then began to decline at a fairly constant rate throughout the rest of the experiment, including the 60-day depuration period. At the low oil level, clams showed a decline in hydrocarbon content during the last 30 days of exposure and a slower rate of decline after exposure to oil ceased. At the low and medium oil levels, clams approached the same hydrocarbon level during the 60-day depuration period. While there was a 100-fold difference in the amount of oil to which the clams were exposed, after Day 60, there was less than a 10-fold difference in hydrocarbon content among the clams exposed to oil.

The concentration of hydrocarbons found in each column chromatographic fraction is summarized in Table 1. Fraction 1 contained saturated hydrocarbons and Fraction 2 aromatic hydrocarbons and biogenic olefins. The control clams contained more Fraction 2 material than Fraction 1 material, as did the clams in the low concentration of oil after Day 60. At the higher concentrations of oil, clams had more Fraction 1 than Fraction 2 material. Chromatograms of a typical oil/water dispersion (Fig. 4 a,b), *Macoma balthica* tissue after 120 days of exposure to oil (Fig. 4 c, d), 180 days of exposure to oil (Fig. 4 e,f), and 60 days of depuration (Fig. 4 g,h) demonstrate that a considerable change occurred in the composition of the oil accumulated by the clams. For the aliphatics, the relative amount of lighter compounds, those in the region bounded by dodecane and hexadecane on the ehromatogram, increased from 25% at 30 days of exposure to 57% after 180 days of exposure (Table 2).

The fractionation of aliphatics within the dodecane to hexadecane range was studied further. Normal alkanes, branched alkanes, monocyclic alkanes and bicyclic alkanes were assayed by GC-MS to determine the relative intensity of parent peaks of each class with 12 to 16 carbon atoms. This was done for extracts of the oil-in-water and oil-exposed clams collected after 90, 180 and 240 days. For each sample, the ion intensity data of the 5 carbon numbers investigated was normalized within each compound class. Then the data were regrouped by carbon number and each compound class was renormalized over the sampling sequence. The double normalization eliminated the effect of variations in relative

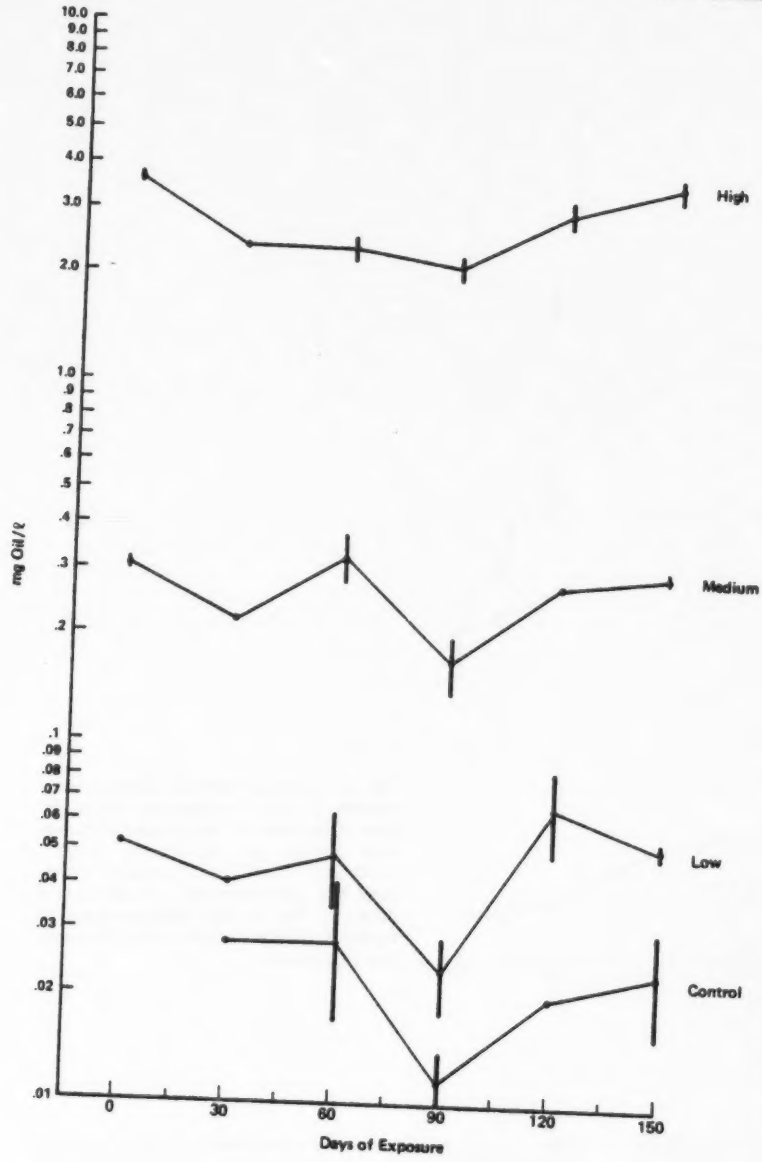

Fig. 2. Concentration of total oil in seawater in test and control tanks during course of experiment. Bars show range of duplicate determinations, dots are single determinations

abundance of the parent ions in the individual mass spectra of compounds in the 4 classes; this procedure demonstrated the enhancement or diminution for each carbon number of each compound class relative to the total abundance in that compound class at any given time. These results for the branched, monocyclic and bicyclic alkanes are shown in Fig. 5. The results for normal alkanes are not shown, since this compound class displayed diminution through time for all chain lengths. The branched alkanes showed relative enhancement of 12 and 13 carbon atom compounds through time and relative diminution of larger compounds. In the monocyclic alkanes, relative enhancement was observed for compounds with 13 and 14 carbon atoms; while the bicyclics showed relative enhancement only for compounds of 14, 15 and 16 carbon atoms.

Aromatic compounds identified and quantified by single-ion MS, and their mean alkyl substitution level are presented in Tables 3 and 4, respectively, for low oil-level clams and the oil/seawater dispersion. Relatively high levels of these compounds were found in clams before the start of the experiment, suggesting that *Macoma balthica* in Resurrection Bay are exposed to low levels of aromatic hydrocarbons. The control clams, after 180 days in clean seawater had lost most of the aromatic compounds, but still retained significant amounts of the anthracenes, fluoranthenes, and dibenzothiophenes (Table 3). (Here and throughout this paper

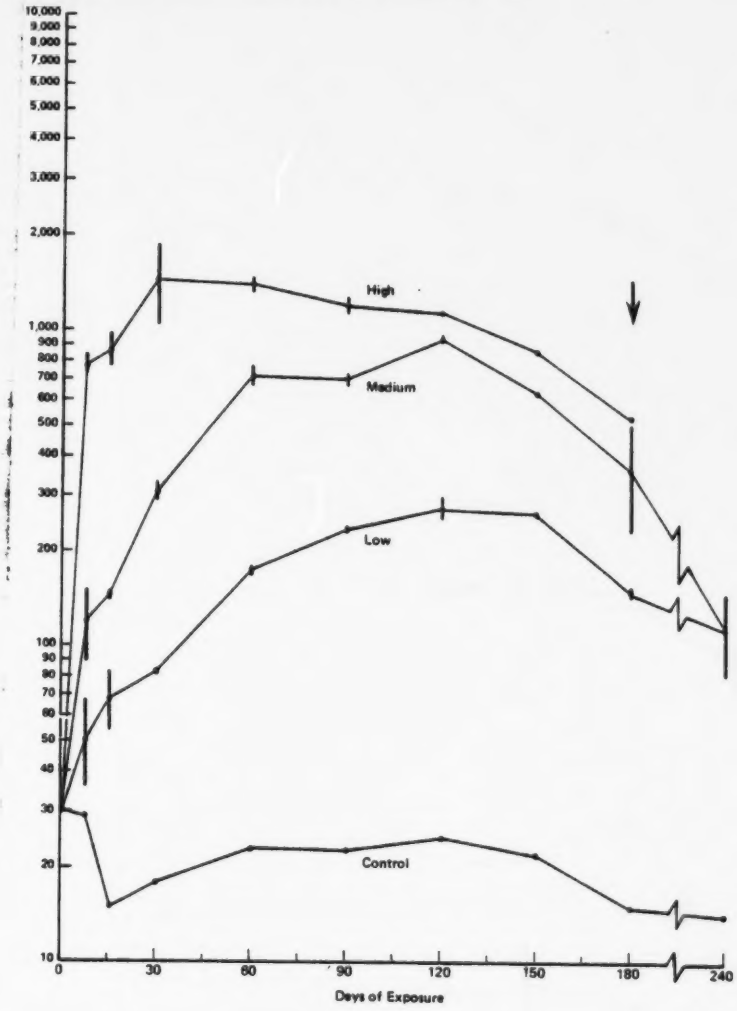

Fig. 3. *Macoma balthica.* Tissue concentrations of total hydrocarbons during 180 days of exposure to oil in seawater dispersions and 60 days of recovery. "High", "medium", "low", and "control" correspond to oil-in-seawater concentrations shown in Fig. 2. Bars indicate range of duplicate determinations; dots are single determinations

we use the name of one aromatic ring system to indicate that system and its isomers: thus "anthracene" means "anthracene and phenanthrene"; and by "anthracenes" we mean "anthracene, phenanthrene and their alkyl homologs".) The mean substitution level for most of the parent compounds generally increased with continued oil exposure (Table 4), and even upon depuration. There was a considerable difference between the mean substitution level of the oil-exposed clams after 60 days depuration and that of the oil/seawater dispersion for all the aromatic systems except the fluoranthenes. In general, there was selective retention of more substituted compounds (Table 5), except the chrysenes for which the mean substitution level was lower after depuration than in the oil/seawater dispersion (Table 4).

The concentration of aromatic compounds determined by GC-MS and the total concentration of hydrocarbons in the second fraction of column chromatog-

Table 1. *Macoma balthica.* Hydrocarbon concentrations ($\mu g\ g^{-1}$ wet weight basis) in tissues during oiling experiment for each column chromatographic fraction (F_1 and F_2). nd: not determined

Day	Treatment							
	Control		0.03 mg l^{-1}		0.3 mg l^{-1}		3.0 mg l^{-1}	
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
0	7.9	22	nd	nd	nd	nd	nd	nd
7	7.7	13	30	22	60	63	480	310
15	8.9	6.4	37	32	80	63	570	310
30	8.2	9.4	48	33	190	120	1020	430
60	9.7	14	91	84	390	340	780	600
90	9.5	13	108	130	350	300	630	570
120	8.8	16	130	140	540	400	630	510
150	9.5	13	150	110	390	250	500	360
180	7.2	8.4	68	81	240	130	160	350
240	6.9	7.4	64	51	61	52	nd	nd

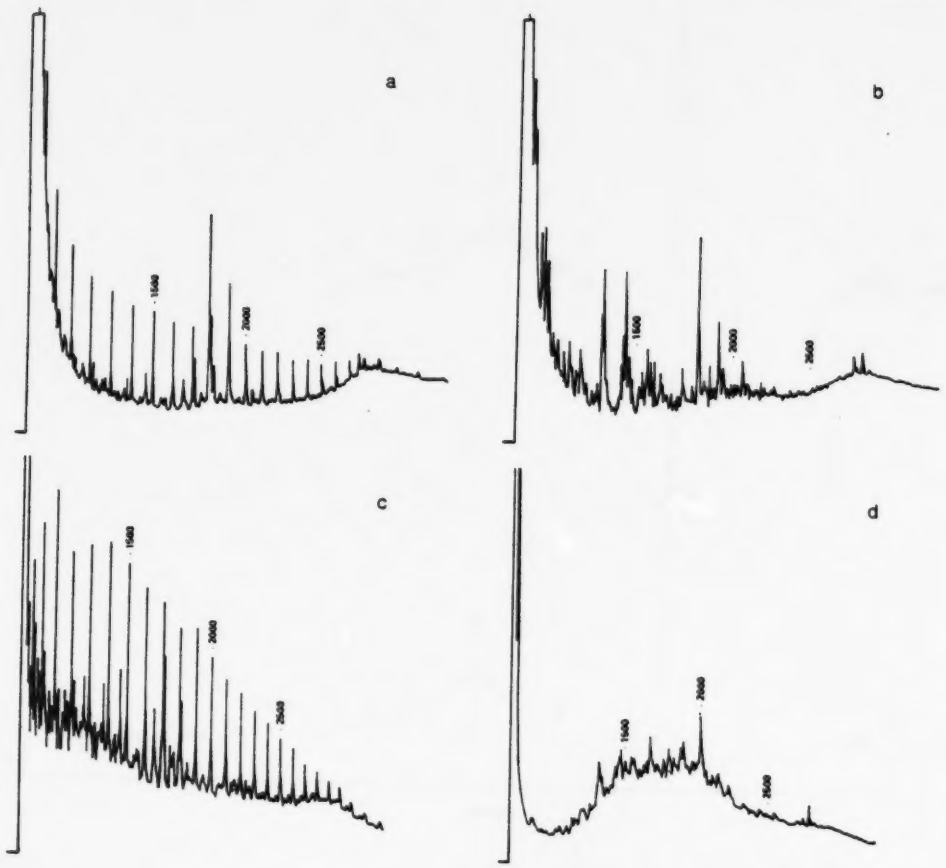

Fig. 4. *Macoma balthica.* Gas chromatograms of oil-in-water dispersion and tissue accumulations of hydrocarbons. (a) Saturate fraction and (b) unsaturate fraction of oil in water dispersion; (c) saturate fraction and (d) unsaturate fraction of tissue after 120 days; (e) saturate fraction and (f) unsaturate fraction of tissue after 180 days; (g) saturate fraction and (h) unsaturate fraction of tissue after 240 days. Retention indices are shown for each chromatogram

Table 2. *Macoma balthica.* Percentage of total saturated aliphatic hydrocarbons eluting between dodecane and hexadecane during gas chromatography

Day	Treatment			
	Control	0.03 mg l^{-1}	0.3 mg l^{-1}	3.0 mg l^{-1}
0	10	nd	nd	nd
30	3	25	30	28
60	5	34	33	32
90	2	49	39	36
120	3	43	32	34
150	1	40	33	30
180	4	57	52	44
240	0.5	53	55	nd

raphy determined by GC were not the same, and the concentration found by GC-MS as a percentage of the total varied from 25% in clams before oil exposure to 6% after depuration. The concentration of hydrocarbons determined by GC-MS is an underestimate, as only the parent compounds were calibrated against standards and increased alkyl substitution causes decreasing response in single ion monitoring. The change in the percentage of the total accounted for by MS may be the result of the different levels of substitution encountered, the presence of hydrocarbon or heteroaromatic systems not investigated by GC-MS, or possibly by the formation of oxygenated metabolites whose column chromatographic behavior is similar to the aromatics.

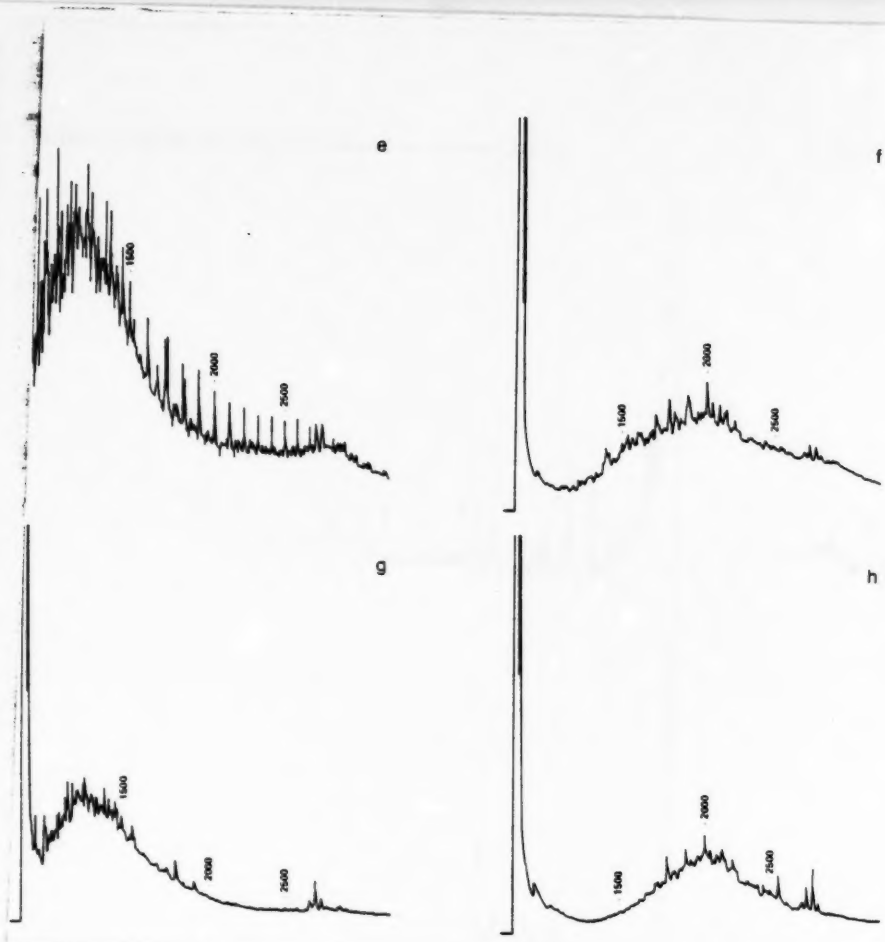

Fig. 4. (continued)

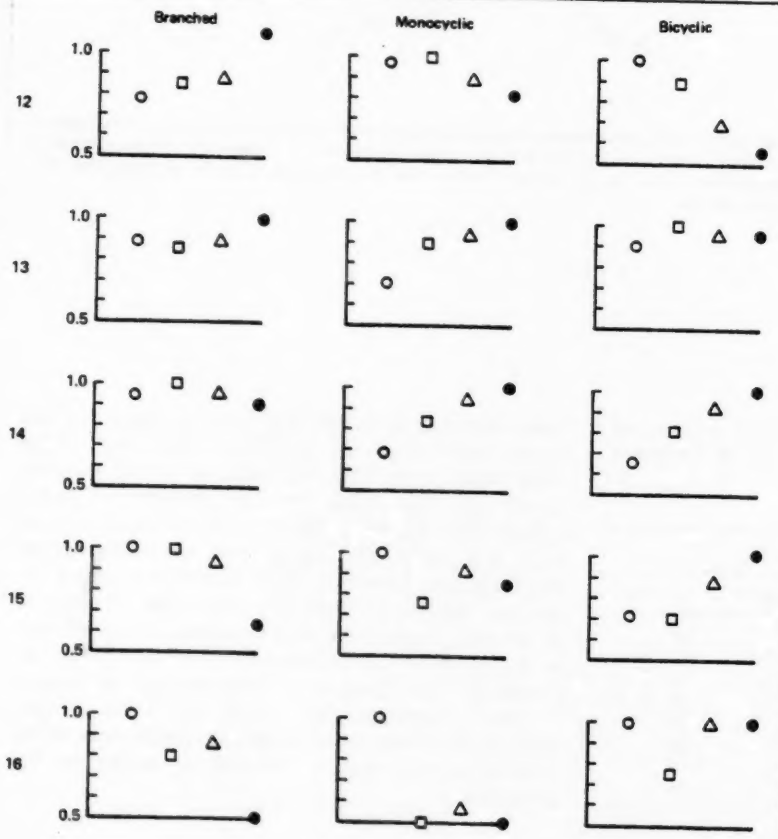

Fig. 5. *Macoma balthica.* Branched, monocyclic, and bicyclic saturated hydrocarbons with 12 to 16 carbon atoms. Each graph in matrix shows amounts of compounds with the indicated structure and carbon number in oiled seawater (open circles), and in tissue at Days 90 (squares), 180 (triangles) and 240 (filled circles) relative to total abundance of structural class at the time. Normalization procedure used to derive these results is described in "Results"

Table 3. *Macoma balthica.* Concentration of aromatic hydrocarbons present in bivalves (ng hydrocarbons g^{-1} wet weight) and exposure water (ng hydrocarbons l^{-1})

Compounds	*Macoma balthica*						Oil/water (0.03 mg l^{-1})
	Control (Day 0)	0.03 mg l^{-1} (Day 60)	0.03 mg l^{-1} (Day 120)	0.03 mg l^{-1} (Day 180)	0.03 mg l^{-1} (Day 240)	Control (Day 180)	
Naphthalenes	120	1600	2400	980	340	21	350
Biphenyls	160	930	1500	780	330	31	86
Tetralins	5.5	640	1000	440	130	0	43
Fluorenes	470	1400	2100	960	260	93	110
Anthracenes	2200	3000	3200	1900	740	380	200
Fluoranthenes	220	920	960	540	200	130	30
Chrysenes	73	310	510	240	68	26	15
Benzothiophenes	0	140	280	120	44	0	14
Dibenzothiophenes	1800	2300	3000	1300	570	130	140
Benzonaphthenothiophenes	150	570	780	470	210	0	24

Table 4. *Macoma balthica.* Mean substitution level (expressed as alkyl carbons per aromatic molecule) of aromatic hydrocarbons present in bivalves and exposure water

Compounds	*Macoma balthica*						Oil/water (0.03 mg l^{-1})
	Control (Day 0)	0.03 mg l^{-1} (Day 60)	0.03 mg l^{-1} (Day 120)	0.03 mg l^{-1} (Day 180)	0.03 mg l^{-1} (Day 240)	Control (Day 180)	
Naphthalenes	5.0	5.2	5.3	6.3	7.2	4.3	3.6
Biphenyls	4.5	5.0	5.0	5.4	5.6	5.6	3.9
Tetralins	6.1	4.8	4.9	5.4	5.7	0	3.4
Fluorenes	3.3	4.7	4.5	4.8	5.6	2.7	3.8
Anthracenes	2.6	3.3	3.3	3.5	3.8	3.5	3.1
Fluoranthenes	1.7	2.4	2.5	2.5	2.4	2.2	2.4
Chrysenes	0.5	1.1	1.2	1.1	0.9	0.7	1.1
Benzothiophenes	0	6.1	5.6	6.1	7.0	0	4.6
Dibenzothiophenes	2.7	3.2	3.2	3.2	3.3	6.0	3.0
Benzonaphthenothiophenes	1.4	1.9	2.0	2.4	2.5	0	1.9

Table 5. *Macoma balthica.* Concentration factor of aromatic compounds in bivalves exposed to 0.03 mg l^{-1} oil in water after 60 days depuration; molecular weights of compounds are given in parentheses. Concentration factor = μg compound g^{-1} wet weight of *M. balthica*/μg compound g^{-1} seawater $\times 10^{-3}$

Alkyl substitution	Naphthalenes	Dibenzothiophenes	Anthracenes	Chrysenes
0	0.05(128)	0 (184)	0.26(178)	4.44(228)
1	0 (142)	0.89(198)	0.55(192)	4.93(242)
2	0.01(156)	2.98(212)	2.82(206)	5.61(256)
3	0 (170)	7.12(226)	5.91(220)	nd
4	0.63(184)	7.96(240)	5.84(234)	nd
5	3.01(198)	8.73(254)	10.56(248)	nd
6	5.40(212)	8.77(268)	10.54(262)	nd
7	9.30(226)	nd	nd	nd

Discussion

The continuous-flow system produced reasonably uniform petroleum exposures at the three test concentrations (Fig. 2). The fluctuations of the measured oil concentrations in the high, medium and low exposures tended to occur together (e.g. all were lowest on Day 90). This is to be expected, since the two lower concentration solutions were produced by dilution of the high concentration.

Previous work has indicated that non-polar lipids partition between animal tissues and the aquatic environment in an equilibrium fashion (Hamelink *et al.*, 1971) and the initial uptake rate of hydrocarbons is directly proportional to the aqueous hydrocarbon concentration (Stegeman and Teal, 1973). Expressed symbolically these relationships are:

$$K = [O_A]/[O_W], \quad (1)$$

$$\text{and } d[O_A]/dt = k_l[O_W], \quad (2)$$

where K is the equilibrium constant for the partition of oil between *Macoma balthica* and the water, $[O_A]$ is the

concentration of oil in the clam, $[O_W]$ is the concentration of oil in water, and k_1 is the rate constant for the initial uptake of oil by the clam. Using our data from Fig. 3, we have evaluated (1) at 120 days and (2) at 7 days for the three values of $[O_W]$. K varied from 9.2×10^3 at 0.03 mg l^{-1} down to 3.8×10^2 as the oil concentration rose to 3.0 mg l^{-1}; and k_1 ranged from 95 per day at the high oil concentration to 34 per day at the low concentration of oil. Both "constants" had intermediate values at 0.3 mg l^{-1} oil. Both k_1 and K increased as $[O_W]$ decreased; this indicates that *M. balthica* is more efficient at extracting and retaining oil from water at low oil-in water concentrations.

One explanation for this observation is that the concentration of hydrocarbons in the clams is related not only to the hydrocarbon concentration in the water, but also to the amount and efficiency of water filtration accomplished by the clams at the various oil-exposure levels. Stainken (1975) has observed the mode of accumulation of an oil/water dispersion in a filter-feeding bivalve (*Mya arenaria*). Oil droplets are processed as food and are accumulated in the gut and digestive diverticula and from there are transferred to the rest of the body, probably as individual molecules. No food was administered to the *Macoma balthica* in the present study for 24 h prior to sampling for hydrocarbon analysis, however the clams were left in seawater containing oil droplets. No attempt was made to segregate the gut from the other tissues; thus, the hydrocarbon data are for entire clams, including gut contents without food. At higher oil concentrations in this experiment, the rate of uptake declined—probably as a result of the greatly reduced siphon activity of the clams exposed to the high oil concentration (Stekoll *et al.*, 1980).

The uptake-rate response of *M. balthica* over the large range of concentrations of this experiment demonstrated that other behavioral patterns in addition to shell closure at very high exposure levels may influence the rate at which hydrocarbons are taken up by organisms.

Bivalves exposed to oil usually fail to return rapidly to pre-exposure hydrocarbon levels during depuration (Lee *et al.*, 1972; Stegeman and Teal, 1973; Clark and Finley, 1975; Fossato, 1975; Boehm and Quinn, 1977). Stegeman and Teal (1973) hypothesized a stable compartment in an organism which, after saturation with hydrocarbons, released those hydrocarbons very slowly. In our experiment, the low- and medium-exposure clams returned to approximately the same hydrocarbon level after 60 days of depuration. It may be that this final oil concentration of 110 mg g^{-1} (wet weight) represents the size of this "stable compartment" for *Macoma balthica*. However, in the absence of information from even longer depuration periods, this conclusion is tentative.

As illustrated in Fig. 4, our experimental work shows that fractionation of Prudhoe Bay crude oil occurred during both accumulation and depuration by *Macoma balthica*. Because of the extreme molecular complexity of the crude oil, we have not examined the fractionation process for individual compounds but have investigated the behavior of several classes of compounds present. Table 1, which records the concentrations of aliphatic and aromatic hydrocarbons in *M. balthica* shows that aromatics were enhanced in the clam's tissues throughout the experiment. The ratio of aliphatics to aromatics (F_1:F_2) in the oil-in-water dispersion was 3.7. However, this ratio in the tissues was generally less than 1.5 and always less than 2.5. Clearly, *M. balthica* selected in favor of aromatics both in initial uptake rate and in equilibrium partition.

Fig. 5 indicates that within the aliphatic fraction, cyclic compounds were generally retained to a greater extent than branched chains which, in turn, were preferentially held over linear alkanes. Similar fractionation has been observed by other workers (e.g. Blumer *et al.*, 1970; Stegeman and Teal, 1973). Fig. 5 and Table 2 also show that molecular size (carbon number) influenced retention. Although these results are too complex for detailed interpretation, it seems reasonable that selective metabolism, tissue transport, membrane transport or final deposition site may be related to the size, solubility or configuration of the compound (Tanford, 1978). The apparent preference for accumulation and retention of lighter aliphatic compounds, (Table 2) was unexpected in view of decreasing aqueous solubility with increasing molecular weight. This may indicate that uptake of aliphatics occurs *via* the dissolved phase, with attendant fractionation, but that release involves some other process.

While the relative percentage of the aromatic ring systems remained fairly constant during the course of our experiment, the mean alkyl substitution level increased, most noticeably in the lighter aromatic compounds. Given the inherent change in sensitivity of MS with increased levels of alkyl substitution, it is difficult to demonstrate a change in relative amount of the sum of each parent compound and its alkyl substituents. Changes in the relative amount of alkyl-substituted aromatic compounds have been observed in a benthic anemone following an oil spill (Grahl-Nielsen *et al.*, 1978). Only aromatic compounds with up to 3 carbon alkyl substitution were examined, but there appeared to be a pattern favoring higher alkyl substitution with time. A field study of *Modiolus demissus* chronically exposed to oil (Lake and Hershner, 1977) demonstrated a loss of lighter aromatic compounds upon depuration and an apparent relative increase in 2,3,6-trimethylnaphthalene, fluoranthene, and pyrene. These authors concluded that most diaromatics are lost upon depuration and that tri- and tetra-cyclic aromatics are retained. When a larger range of alkyl-substituted compounds is available, as in the present study, a pattern of increasing retention with increasing molecular weight emerges, both among unsubstituted aromatic compounds and among various alkyl substitutions of any given aromatic ring system. However, for compounds of a given molecular weight, the more substituted one has a higher concentration factor (Table 5). Thus, in contrast to the behavior of aliphatic hydrocarbons, retention of aromatic compounds appears to decrease with increasing aqueous

solubility, and the long period of slow depuration observed in many bivalves may result from relatively high concentrations of less water-soluble aromatic compounds.

In studies of aromatic sulfur compounds in organisms upon recovery from oil exposure, Grahl-Nielsen *et al.* (1978) found an increase in the relative amount of dibenzothiophene and its alkyl derivatives, whereas Lake and Hershner (1977) found a decrease in aromatic sulfur compounds that paralleled the decrease in aromatic compounds as a whole. In the present study there was little or no enhancement of the aromatic sulfur compounds relative to other aromatic compounds. The concentration factor for C_6-substituted dibenzothiophenes (Table 5) fell between C_6 naphthalenes and C_6 anthracenes, about where one might expect it on the basis of molecular weight.

It thus appears from our laboratory data, that when *Macoma balthica* is exposed to chronic low levels of an oil-in-water dispersion, the characteristics of the oil are extensively modified within the clam. The aliphatic compounds are handled quite differently than the aromatics. We have suggested selective metabolism as one of the possible causes of fractionation of petroleum by *M. balthica*. Although much work under short-term acute conditions (Varanasi and Malins, 1977) indicates that molluscs have little or no ability to metabolize hydrocarbons, we believe that the presently available information does not exclude the possibility of selective metabolism during longer accumulation and release periods.

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